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Puncture versus capture: which stresses animals the most?

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Abstract

The prerogative of animal welfare science includes wild species and ecological studies. Yet, guidance enshrined in legislation is narrowly derived from studies involving laboratory rodents; legitimacy for non-mammalian free-ranging species is thus debatable. The European directive 2010/63/EU illustrates this problem. It includes this key statement: "Practices not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle…" which determines if the directive shall apply. Protocols involving surgery clearly fall within the scope of the directive: procedures are scrutinized, investigators and technicians must be qualified and various agreements are required (e.g. issued by an ethical committee). By contrast, non-invasive procedures, like mark-recapture population studies, merely need a permit from wildlife authorities (at least in most countries). Yet, blood sampling that implies the introduction of a needle—one of the most common practices in animals—could shift any study on the constraining-side of the directive, on the grounds that puncture impacts individuals more severely than capture. We examined the validity of the needle-threshold using the stress response of free-ranging snakes. Our results based on physiological markers show that blood sampling does not add any stress to that triggered by capture, and thus questions the usefulness of the needle-threshold to gauge welfare in wild animals. The specificities of studying wild species should be considered to redress captivity biased animal welfare policy.

Keywords Animal welfare · Blood sampling · Corticosterone · Glucose · Reptile · Stress markers

Introduction

Research in animal welfare science addresses a wide range of topics and aims to influence practices and legislation. In addition to ethical considerations, adopting the best practices to promote the wellbeing of the animals used for farming or scientific research can enhance the quality of the outcomes (e.g. agricultural production, the accuracy of scientific results), participate in the protection of wildlife and ecosystems and can even contribute to the amelioration of public health (Minteer and Collins 2005; Broom et al. 2013; Goldberg 2016; Pandolfi et al. 2017). However, fully implementing animal welfare into legislation, training or educational programs is often a daunting challenge; resistances come from all sides (Clippinger et al. 2016; Dawkins 2017; Miranda-De La Lama et al. 2017; McCulloch and Reiss 2018). Part of the inertia has a historical background; conceptual, technical and knowledge obstacles are important too (Fraser 1999; Burghardt 2009; Franco 2013; Sneddon et al. 2018).

In the nineteenth century, maltreated horses used to power transports triggered the creation of societies for the protection of animals, and accordingly, induced improvements of the law (e.g. SPCA in 1824, spcai.org). Recently, the ban of animal-tested cosmetics in the European market prompted the development of alternative approaches (Adler et al. 2011). In contrast, initiatives to take into account the sufferance of animals used in food industries have been stifled by business priorities, limiting progress and eventually enabling the worldwide legal proliferation of inhumane battery farming with tragic environmental consequences (Tsiafouli et al. 2015). On the other hand, guidance for ethical

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experimentations of laboratory mammals has been developing for more than 70 years (Russell and Burch 1959) and is continuously ameliorating scientific procedures (Knight et al. 2009; Franco 2013). However, its generalization to non-mammalian lineages is still in its infancy (Lindsjö et al. 2016; Zemanova 2017; Sneddon et al. 2018). Overall, the situation of tens of billions of animals that are predominantly used for agricultural production every year, and secondarily for scientific and other purposes, remains questionable (Broom et al. 2013; Mitchell 2011).

During the past decades, growing worries on the part of the general public about animal welfare has prompted political reactions. A number of regulations are unsatisfactory however. Salient examples are provided by the European DIRECTIVE 2010/63/EU (2010). At present, this directive includes only vertebrates and cephalopods (Drinkwater et al. 2019). Therefore, the vast majority of lineages and species are excluded; notably arthropods, supposedly because they have no capacity to feel pain. Yet, abundant studies have demonstrated that these organisms are fully equipped to perceive nociceptive stimuli and are able to express specific avoidance behaviours, thereby indicating that they can feel pain (Sneddon et al. 2014). This later topic is controversial however (Adamo 2019), illustrating the difficulty to frame into a consensual approach the disparity of the criteria used to assess animal welfare (Fraser 1999). We are at a pivotal period where objective information is required to assist regulations.

Whatever the case, vertebrates (at least at post larval stages) are covered by precisely defined guidance and official decrees. In this context, blood sampling, one of the most common practices in animal studies, occupies a central position. It is precisely here that we may determine the degree of invasiveness and suffering that can be inflicted to animals, under which the legislation for the protection and welfare of animals used for scientific purposes does not apply and for which ethical committee agreement is generally not required. Established for laboratory animals (mostly rodents), the DIRECTIVE 2010/63/EU (2010) stipulates that "practices not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice" do not fall within the scope of regulated procedures. Undoubtedly, this threshold represents a significant improvement for experimental studies that involve captive vertebrates (Ricceri and Vitale 2011). Its applicability in wild animals has not been assessed, but the rule applies equally both to captive and wild animals. There is a tradeoff between the legitimacy and the applicability of laws, justifying taxonomic shortcuts (i.e. the generalized nature of legislation has drawn conclusions that are applied in a broad taxonomic context without sufficient evidence). Testing the worth of the needle-threshold in field studies is nevertheless essential, notably to assist further technical and legislation improvements.

In the current study we address this key issue regarding wild animals. Yet, how can we evaluate the specific impact of puncture in terms of animal welfare? In vertebrates, painful or threatening events cause a rapid and massive release of stress hormones, chiefly catecholamines and glucocorticoids, in the bloodstream (Romero 2004). Assessing changes of plasma concentrations of these hormones is a routine technique to gauge the impact of stressful events in wild animals (Romero 2004; Mormède et al. 2007). Acute stress responses are influenced by the type of stressors employed and by their intensity (Skoluda et al. 2015). Thus, we hypothesize that if the introduction of a needle is effectively painful and harmful, an acute response should be detected with physiological markers (Mormède et al. 2007).

However, capture and restraint of the subject are inevitable before puncture. Consequently, the stress occasioned by the introduction of a needle per se cannot be dissociated from the stress provoked by handling. We circumvented this difficulty by relying on the dynamic aspect of the stress response. We hypothesized that a possible additional stress of puncture over capture stress would provoke a greater release of stress hormones compared to handling alone. Because circulating hormones are not cleared immediately, a possible carry-over effect caused by the penetrating needle should result in higher plasma levels measured during subsequent samplings. Consequently, comparing the trajectories of stress hormone levels during acute stress responses in individuals subjected—or not—to initial puncture and subsequently monitored with repeated blood samples, provides a means to test if the needle-threshold is an adequate welfare criterion in free-ranging animals.

Material and methods

Experimental design

The main objective was to detect possible strong stress (or distress?) caused by the introduction of a needle that is greater than the stress caused by handling per se (Fig. 1). It is essential to start from basal levels; thus, the subjects should not detect the observers from distance before capture. Next, catching and sampling blood must be rapid in order to keep precise timing across subsequent blood samples and to minimize the possible impact of handling before the blood is effectively collected. A system where animals can be very rapidly collected and easily sampled offers the desired technical advantages. Trapping or chasing animals and complex restraining procedures are consequently inappropriate (Johnstone et al. 2012).

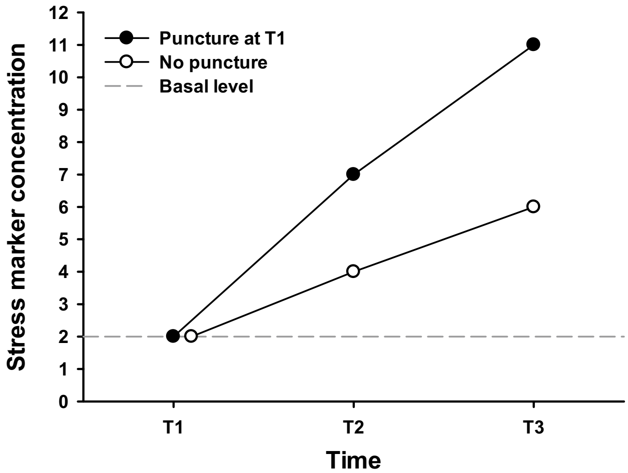

Fig. 1 Hypothetical scenario and data used to tackle the needle threshold problem. Handling can cause an elevation of stress hormone concentration (open circles). This effect, classically observed in capture–mark–recapture studies, is assumed to be acceptable in terms of animal welfare and is not covered by the European directive 2010/63/EU. By contrast, puncture (introduction of a needle during blood sample in the field for example) presumably triggers a strong stress (black circles) that requires the application of complex regulations to ensure that official guidance (e.g. 3Rs) is respected. Mean levels of stress marker concentration are arbitrary

For these reasons we selected a dense population of animals that can be easily manipulated and sampled, the dice snakes *Natrix tessellata* (Ajtić et al. 2013). Adult individuals were captured in July 2018 on the shores of Golem Grad Island (18 ha, Prespa Lake, North Macedonia). The snakes were visually searched and rapidly seized by hand; approximately 100–150 µL of blood was rapidly taken by cardiocentesis using 1.0 mL syringes with 30G heparinized needles. The blood was effectively collected 3.2 ± 1.5 min (± SD) after capture. The snakes were then kept in a calico bag (laid in the shade) for repeated blood sampling 15 min (15.8 ± 1.4 min) and 30 min (31.3 ± 2.0 min) later. On average, the total amount of blood collected per individual represented less than 0.15% of snake body mass (~ 0.12 ± 0.09%, range 0.05–0.47%). All the snakes were measured after the last puncture and released at the place of capture. None of the snakes exhibited any sign of distress or disorder at release.

Snakes were randomly allocated to one of the two treatments: initial puncture *vs* not (Fig. 1). In the puncture group they were rapidly sampled ($N = 13$ snakes bled 3 times, at t1, t2 and t3). In the control group, the snakes were handled in the same manner as snakes that were punctured but the initial puncture was skipped ($N = 17$ snakes bled twice, at t2 and t3). In this latter group, a subgroup of four snakes were further manipulated as if they were blood sampled: rapidly after capture, they were placed on their back and the skin above the heart was gently touched with a small stick for 30 s (i.e. mimicking a blood sample without introducing a needle). In practice we found no difference in stress responses between the subgroups ($N = 13$ initial puncture skipped vs $N = 4$ initial puncture mimicked); therefore, they were pooled. Placing individuals on their back, inserting the needle and taking blood (or mimicking this step) was rapidly performed (~ 1 min) and represented a small fraction of total handling time (e.g. ~ 3 min at t1) compared to capture plus other manipulations (transport from capture to the place of sampling, putting snake into a bag…). The total number of blood samples was $N = 73$ ($N = 13$ at t1, $N = 30$ at t2 and $N = 30$ at t3). Sex sample sizes were as follows: 19 females (11 control + 8 punctured at t1) and 11 males (6 control and 5 punctured at t1). All procedures were approved by North Macedonia and French authorities (permits 03-246, 09/346/DEROG, A79-001, and 79-157).

Stress markers

Blood glucose concentration (GLUC) was immediately evaluated with a drop of blood (5 µL) using miniature glucometers (Bayer Contour Next/One). The rest of the blood was then centrifuged at 6000 rpm (3 min); the plasma was collected in 1.5 mL cryotubes and stored in liquid nitrogen. Plasma corticosterone level (CORT) was measured using radioimmunoassay in the CEBC laboratory (see Bonnet et al. 2013 for details).

Statistical analyses

CORT values were Box-Cox transformed to meet the normality assumption (Shapiro–Wilk $W = 0.977$, $P = 0.197$). The distribution of the other continuous variables did not deviate from normality. The assessment of stress responses involved a design with a repeated (within-subject) factor: GLUC or CORT repeated over time for each individual in this study. We analysed GLUC and CORT responses separately. Therefore, we used a general linear model (GLM) with a single repeated factor, either GLUC or CORT, and puncture treatment as the main categorical predictor. Possible interactions with sex (another categorical predictor) were examined and body mass was implemented as a covariate. The distribution of within-cell residuals revealed that the plots of expected normal vs observed values followed almost linear patterns without outlier. Analyses were performed with Statistica 13.5.0.17.

Results

Both markers, GLUC and CORT responded strongly with a sharp increase over time revealed by highly significant effect of time in the GLM for repeated measures

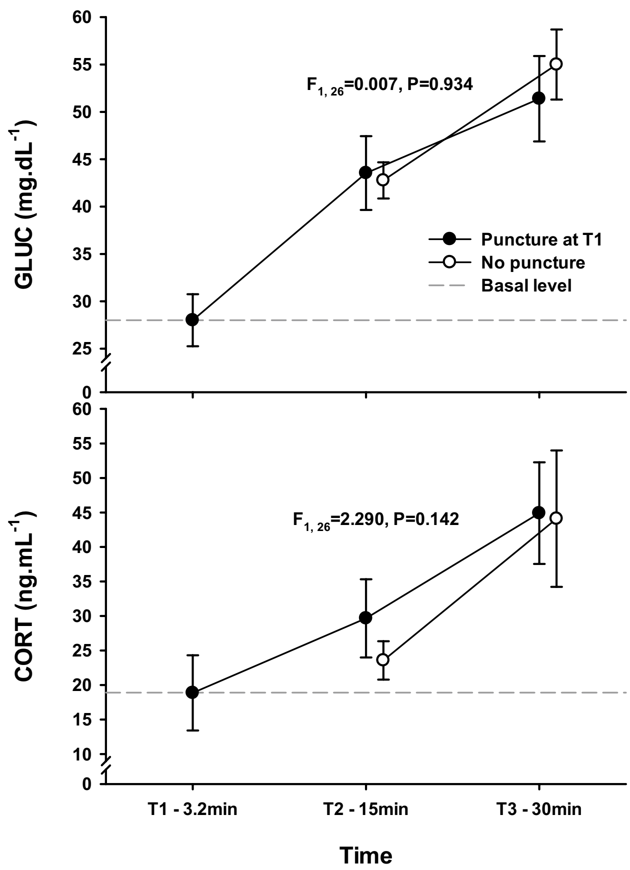

Fig. 2 Glycaemia (GLUC, mean ± SE, mg dL^{-1}) and corticosterone level (CORT, mean ± SE, ng mL^{-1}) were measured in two groups of snakes (i.e. treatment effect in Tables 1, 2). In one group (Puncture at T1; black circles $N = 13$), the snakes were sampled three times. The first puncture was omitted in the second group (No puncture; open circles, $N = 17$), the snakes were sampled twice at T2 and T3. This provided a mean to compare the effect of the initial puncture (T1) on the following stress responses of the groups at T2 and T3. Both GLUC and CORT increased after capture, but a possible additional effect of puncture was not detected: the trajectories were not different between the two groups

Table 1 Summary of the GLM analyses where repeated measures, 15 min and 30 min after capture, of blood glucose levels (GLUC, time effect) were compared between individuals that experienced—or not—the introduction of a needle at capture (treatment)

Effect	SS	*df*	*F*	*P*
Intercept	12,4392.9	1	375.514	< 0.001
Sex	335.5	1	1.013	0.324
Treatment	75.1	1	0.227	0.638
Sex × treatment	230.2	1	0.695	0.412
Error	8612.8	26		
Time	1514.0	1	36.332	< 0.001
Time × sex	41.2	1	1.000	0.329
Time × treatment	71.9	1	1.725	0.201
Time × sex × treatment	0.3	1	0.007	0.934
Error	1083.5	26		

In addition to this factor, sex was implemented. GLUC increased sharply over time, but without interaction with sex or treatment (Fig. 2)

Table 2 Summary of the GLM analyses where repeated measures, 15 min and 30 min after capture, of plasma corticosterone levels (CORT, time effect) were compared between individuals that experienced—or not—the introduction of a needle at capture (treatment)

Effect	SS	*df*	*F*	*P*
Intercept	2992.7	1	296.840	< 0.001
Sex	54.7	1	5.423	0.028
Treatment	1.4	1	0.141	0.711
Sex × treatment	7.9	1	0.787	0.383
Error	262.1	26		
Time	49.1	1	29.351	< 0.001
Time × sex	3.6	1	2.177	0.152
Time × treatment	0.36	1	0.212	0.649
Time × sex × treatment	3.8	1	2.290	0.142
Error	43.5	26		

In addition to this factor, sex was implemented. CORT increased sharply over time, but without interaction with sex or treatment. A significant univariate effect of sex became non-significant when the effect of time was taken into account

(Fig. 2, Tables 1, 2). The response occurred earlier for GLUC compared to CORT, however: Tukey post-hoc tests revealed a significant difference between t1 and t2 for GLUC ($P < 0.002$, $N = 13$), but not CORT ($P = 0.1461$, $N = 13$).

For both stress markers, there was no difference between the trajectories of the two experimental groups (Tables 1, 2). Body mass and sex did not contribute significantly to the acute response. The broad marginal effect of sex on CORT (Table 2) was due to slightly higher values in males; it became non-significant when the effect of time was taken into account (Table 2).

Discussion

The fast sampling procedure likely minimized stressful effects before the blood was actually retrieved, allowing obtaining accurate basal levels. This condition was essential to isolate the specific impact of the introduction of a needle (Fig. 1). We did not find any evidence for such needle impact, however. In contrast to laboratory animals, wild vertebrates are not accustomed to human observers; thus, even a gentle capture can be assimilated to a predation event that triggers strong stress response that may mask other effects. The strong acute stress responses without any additional puncture effect were thus predictable. Yet, this study is the first to examine this issue in a natural setting and additional stress provoked by blood sampling was fully imaginable. Below we discuss eco-physiological aspects and consider legislative framework.

Eco-physiological perspective

For any wild animal, escaping predation is a priority. Once captured, the snakes reacted vigorously, displaying anti-predator behaviours (shaking, hissing, defecating…). A possible puncture effect was marginal in this context. The penetration of the needle involved modest histological impact (i.e. limited tissue destruction or loss). Indeed, we used very small needles (30 G; 0.3 mm diameter) and we rapidly retrieved small amounts of blood (< 0.15% of snake mass). Serial cardiocentesis performed in young pythons, 39 blood samples per individual, using relatively large needles (25 G–22 G; 0.5–0.7 mm diameter) has shown that this technique is well-tolerated and causes none or minimal impact to the cardiac tissues (Isaza et al. 2004). A fortiori, 2–3 punctures with a much smaller needle would entail negligible risks of harming the snakes.

Animals seized by a large predator launch all their defence mechanisms; a process notably underpinned by the fast activation of the hypothalamo-pituitary-adrenal axis (Goldstein 2003). The hypothalamus rapidly integrates visual (i.e. the sight of human predators), tactile and proprioceptive (i.e. due to capture restraint) stimuli and triggers a powerful response (Silva et al. 2013; Tovote et al. 2016). The early and massive release of glucose likely resulted from the almost immediate upsurge of plasma catecholamine levels caused by the activation of adreno-medullary hormonal system that participates in pain, panic and frightened emotional states (Yokoo et al. 1990; Goldstein 2003). The slightly delayed surge of glucocorticoids was probably caused by the activation of the hypothalamo-pituitary-adrenocortical system, involved in the mobilization of energetic resources, notably glucose, that sustain the stress response (Romero 2004).

Regardless, a central question for animal welfare was to evaluate the noxiousness of the stress provoked by capture plus blood sampling: will bled individuals suffer illness and prolonged discomfort over days or weeks after release? Increasing stress hormone levels are sometimes inappropriately considered as an index of pain or sickness (Goldstein 2003). There is now general agreement that an acute stress response, although provoking discomfort or distress, is unlikely to cause disease, while a chronic overload of the stress system can be the source of pathologies (Romero et al. 2009). Chronic stress disorders are frequent in modern human societies (Chrousos 2009); but in healthy individuals, sporadic acute stresses are not more harmful than a sprint. Stress induced by blood sampling is not necessarily noxious. Yet, sampling has to be fast, gentle and the volume of blood retrieved must remain low relative to body mass (Brown and Brown 2009). More generally, acute stress response prompted by field procedures (e.g. capture, forced regurgitation) did not impact sea snakes monitored during long-term field studies (Fauvel et al. 2012).

Overall, in wild snakes, specific pain and/or acute stress caused by the introduction of a needle to take blood may be negligible or may be masked (hence is not superior to capture stress), and possible pathological effects are unlikely. Most population studies regarding the impact of blood sampling were performed in birds (reviews in Sheldon et al. 2008 and Voss et al. 2010). The general outcome is that blood sampling has no major detrimental impact in wild birds, although in sparrows both negative and null results have been reported (Brown and Brown 2009; Smith et al. 2016). In contrast, non-invasive techniques like banding or fitting individuals with data loggers can compromise flight due to increased drag and thus can deteriorate survival and body condition (Putman 1995; Saraux et al. 2011; Costantini and Møller 2013). Detrimental effects of bio-logging have also been documented in sea-turtles and marine mammals (McIntyre 2015; Hamelin and James 2018). Moreover, capture can provoke tail loss in 10% of several species of lizards and reduce their fitness (Scroggie and Clemann 2009). Field researchers are constantly improving monitoring techniques and efficaciously minimizing their impact on wild animals. The vast majorities of field studies are harmless to populations (Clewley et al. 2018; Swierk and Langkilde 2018). Available information suggests that blood sampling is not the priority in terms of animal welfare.

Legal context

Why then, is the needle-threshold inscribed in legalisation? The introduction of a needle in a blood vessel, although a routine benign procedure, is often rated as painful by humans and captive animals; this amply justifies ethical considerations (Lee and Goosens 2015). The strong symbolic and emotional significations of blood for humans may also have played a role (Carsten 2011). But anthropomorphic criteria may not be able to apply as equally as well to optimize research procedures with regards to wild animals as it does to captive ones (Langkilde and Shine 2006). The official position that non-invasive techniques used in population studies (e.g. trapping, handling, marking) are mild compared to blood sampling does not match eco-physiological evidence. Capture and restraint represent major events for wild animals.

Should we stop field studies altogether?

A claim voiced by a growing number of non-governmental organizations, the total cessation of the use of animals for scientific purposes, is officially enshrined as a long-term objective in European laws. Legislators have ignored the everyday life of wild animals. Only death can reduce stress levels to zero. Setting up artificial anthropogenically biased moral frontiers will drive regulations up a blind alley without any net benefit to wild animals. It is essential to move away from such unrealistic views to one based on practical biologically informed ethical facts. Field studies and their plethora of techniques (blood sampling, bio-logging, biopsies, etc.) are vital to better understand how animals live, for educational programs, or to assist conservation actions. Sound regulations should be set up within the scope of enlightened policies to stimulate researchers to pursue their efforts to better define all procedures. Minimizing short-term stress imposed on individuals from any lineage where nociception has been documented is ethically crucial.

Questioning wild animals about their feelings is almost impossible, however. Many animal species do not exhibit any signs that can be easily monitored in order to distinguish discomfort, panic or distress. This is the case, for example, for a tortoise with its head and legs withdrawn into its shell. Physiological assessment can be useful to skirt this problem: acute stress response and chronic stress monitoring notably.

Conclusion

The 2010/63/EU directive is a comprehensive text that encompasses a wide array of topics and that successfully places animal welfare questions at the heart of procedures. It cannot be summarized with the needle-threshold criterion. On the other hand, blood sampling is widely used in the field for a wide range of eco-physiological, sanitary and genetic investigations. Our study has shown that the effect of a needle puncture is negligible in comparison to the effect of capture and manipulation and that it does not cause additional distress. Further studies are certainly needed in other lineages (e.g. birds, mammals) to test the validity of our results. Yet, the likelihood that dice snakes are the only species where capture inflicts a greater stress than blood sampling per se is low; thus we hypothesise that this effect is widespread in wild vertebrates. If so, current cursors could be shifted; for example, routine blood sampling could be included in the pool of harmless procedures. On the other hand, the impact of field studies on individuals and populations should be monitored on a regular basis to allow meta-analyses. Indeed, available information is sparse for most taxa, especially regarding demographic traits, hampering the possibility to identify the best approaches and refine protocols (McIntyre 2015).

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References

Adamo SA (2019) Is it pain if it does not hurt? On the unlikelihood of insect pain. Can Entomol. https://doi.org/10.4039/tce.2019.49

Adler S, Basketter D, Creton S, Pelkonen O et al (2011) Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010. Arch Toxicol 85:367–485

Ajtić R, Tomović L, Sterijovski B, Crnobrnja-Isailović J, Djordjević S, Djurakić M, Golubović A, Simović A, Arsovski D, Andjelković M, Krstić M, Sukalo G, Gvozdenović S, Aïdam A, Michel CL, Ballouard JM, Bonnet X (2013) Unexpected life history traits in a very dense population of dice snakes. Zoologischer Anzeiger 252:350–358

Bonnet X, Fizesan A, Michel CL (2013) Shelter availability, stress level and digestive performance in the aspic viper. J Exp Biol 216:815–822

Broom DM, Galindo FA, Murgueitio E (2013) Sustainable, efficient livestock production with high biodiversity and good welfare for animals. Proc R Soc B 280:20132025

Brown MB, Brown CR (2009) Blood sampling reduces annual survival in cliff swallows (*Petrochelidon pyrrhonota*). Auk 126:853–861

Burghardt GM (2009) Ethics and animal consciousness: How rubber the ethical ruler? J Soc Issues 65:499–521

Carsten J (2011) Substance and relationality: blood in contexts. Annu Rev Anthropol 40:19–35

Chrousos GP (2009) Stress and disorders of the stress system. Nat Rev Endocrinol 5:374–381

Clewley GD, Robinson RA, Clark JA (2018) Estimating mortality rates among passerines caught for ringing with mist nets using data from previously ringed birds. Ecol Evol 8:5164–5172

Clippinger AJ, Hill E, Curren R, Bishop P (2016) Bridging the gap between regulatory acceptance and industry use of non-animal methods. ALTEX Altern Anim Exp 33:453–458

Costantini D, Møller AP (2013) A meta-analysis of the effects of geolocator application on birds. Curr Zool 59:697–706

Dawkins MS (2017) Animal welfare and efficient farming: is conflict inevitable? Anim Prod Sci 57:201–208

DIRECTIVE 2010/63/EU (2010) Directive on the protection of animals used for scientific purposes. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02010L0063-20190626

Drinkwater E, Robinson EJ, Hart AG (2019) Keeping invertebrate research ethical in a landscape of shifting public opinion. Methods Ecol Evol 10:1265–1273

Fauvel T, Brischoux F, Briand MJ, Bonnet X (2012) Do researchers impact their study populations? Assessing the effect of field procedures in a long-term population monitoring of sea kraits. Amphib Reptil 33:365–372

Franco N (2013) Animal experiments in biomedical research: a historical perspective. Animals 3:238–273

Fraser D (1999) Animal ethics and animal welfare science: bridging the two cultures. Appl Anim Behav Sci 65:171–189

Goldberg AM (2016) Farm animal welfare and human health. Curr Environ Health Rep 3:313–321

Goldstein DS (2003) Catecholamines and stress. Endocr Regul 37:69–80

Hamelin KM, James MC (2018) Evaluating outcomes of long-term satellite tag attachment on leatherback sea turtles. Anim Biotelem 6:18. https://doi.org/10.1186/s40317-018-0161-3

Isaza R, Andrews GA, Coke RL, Hunter RP (2004) Assessment of multiple cardiocentesis in ball pythons (*Python regius*). JAALAS 43:35–38

Johnstone CP, Reina RD, Lill A (2012) Interpreting indices of physiological stress in free-living vertebrates. J Comp Physiol B 182:861–879

Knight S, Vrij A, Bard K, Brandon D (2009) Science versus human welfare? Understanding attitudes toward animal use. J Soc Issues 65:463–483

Langkilde T, Shine R (2006) How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. J Exp Biol 209:1035–1043

Lee G, Goosens KA (2015) Sampling blood from the lateral tail vein of the rat. JoVE 99:e52766

Lindsjö J, Fahlman Å, Törnqvist E (2016) Animal welfare from mouse to moose -implementing the principles of the 3Rs in wildlife research. J Wildl Dis 52:S65–S77

McCulloch S, Reiss M (2018) A proposal for a UK ethics council for animal policy: the case for putting ethics back into policy making. Animals 8:88

McIntyre T (2015) Animal telemetry: tagging effects. Science 349:596–597

Mitchell L (2011) Moral disengagement and support for nonhuman animal farming. Soc Anim 19:38–58

Minteer BA, Collins JP (2005) Ecological ethics: building a new tool kit for ecologists and biodiversity managers. Conserv Biol 19:1803–1812

Miranda-De La Lama GC, Estévez-Moreno LX, Sepulveda WS, Estrada-Chavero MC, Rayas-Amor AA, Villarroel M, María GA (2017) Mexican consumers' perceptions and attitudes towards farm animal welfare and willingness to pay for welfare friendly meat products. Meat Sci 125:106–113

Mormède P, Andanson S, Aupérin B, Beerda B, Guémené D, Malmkvist J, Manteca X, Manteuffel G, Prunet P, van Reenen CG, Richard S, Veissier I (2007) Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiol Behav 92:317–339

Pandolfi F, Stoddart K, Wainwright N, Kyriazakis I, Edwards SA (2017) The 'real welfare' scheme: benchmarking welfare outcomes for commercially farmed pigs. Animal 11:1816–1824

Putman RJ (1995) Ethical considerations and animal welfare in ecological field studies. Biodivers Conserv 4:903–915

Ricceri L, Vitale A (2011) The law through the eye of a needle. EMBO Rep 12:637–640

Romero LM (2004) Physiological stress in ecology: lessons from biomedical research. Trends Ecol Evol 19:249–255

Romero LM, Dickens MJ, Cyr NE (2009) The reactive scope model - a new model integrating homeostasis, allostasis and stress. Hormon Behav 55:375–389

Russell WMS, Burch RL (1959) The principles of humane experimental technique, vol 238. Methuen, London. https://altweb.jhsph.edu/pubs/books/humane_exp/het-toc#

Saraux C, Le Bohec C, Durant JM, Viblanc VA, Gauthier-Clerc M, Beaune D, Park YH, Yoccoz NG, Stenseth NC, Le Maho Y (2011) Reliability of flipper-banded penguins as indicators of climate change. Nature 469:203–206

Scroggie MP, Clemann N (2009) Handling-related tail loss in an endangered skink: incidence, correlates and a possible solution. J Zool 277:214–220

Sheldon LD, Chin EH, Gill SA, Schmaltz G, Newman AE, Soma KK (2008) Effects of blood collection on wild birds: an update. J Avian Biol 39:369–378

Silva BA, Mattucci C, Krzywkowski P, Murana E, Illarionova A, Grinevich V, Canteras NS, Ragozzino D, Gross CT (2013) Independent hypothalamic circuits for social and predator fear. Nat Neurosci 16:1731–1733

Skoluda N, Strahler J, Schlotz W, Niederberger L, Marques S, Fischer S, Thoma MV, Spoerri C, Ehlert U, Nater UM (2015) Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity. Psychoneuroendocrinology 51:227–236

Smith RJ, Benson TJ, Carey M (2016) Blood sampling does not negatively affect apparent survival, recapture probability, or within-season site fidelity in Field Sparrows (*Spizella pusilla*). Auk 134:240–246

Sneddon LU, Elwood RW, Adamo SA, Leach MC (2014) Defining and assessing animal pain. Anim Behav 97:201–212

Sneddon LU, Lopez-Luna J, Wolfenden DC, Leach MC, Valentim AM, Steenbergen PJ, Bardine N, Currie AD, Broom DM, Brown C (2018) Fish sentience denial: Muddying the waters. Anim Sentience 3:1

Swierk L, Langkilde T (2018) Does repeated human handling of study animals during the mating season affect their offspring? J Exp Zool A 329:80–86

Tovote P, Esposito MS, Botta P, Chaudun F, Fadok JP, Markovic M, Wolff SBE, Ramakrishnan C, Fenno L, Deisseroth K, Herry C, Arber S, Lüthi A (2016) Midbrain circuits for defensive behaviour. Nature 534:206–2012

Tsiafouli MA, Thébault E, Sgardelis SP et al (2015) Intensive agriculture reduces soil biodiversity across Europe. Glob Change Biol 21:973–985

Voss M, Shutler D, Werner J (2010) A hard look at blood sampling of birds. Auk 127:704–708

Yokoo H, Tanaka M, Yoshida M, Tsuda A, Tanaka T, Mizoguchi K (1990) Direct evidence of conditioned fear-elicited enhancement of noradrenaline release in the rat hypothalamus assessed by intracranial microdialysis. Brain Res 536:305–308

Zemanova MA (2017) More training in animal ethics needed for European biologists. Bioscience 67:301–305